

REMARKS

Please substitute the attached substitute specification, claims and drawings for the present patent application. Prosecution and consideration of the above-identified application is respectfully requested.

Claims 1-7 were cancelled. New claims 8-26 are added. Claims 8-26 are in the case and are before the Examiner.

The claims were rewritten to conform to standard U.S. practice, eliminating multiple dependencies. The claim amendments draw their support from throughout the application as filed, and for specific example, from the claims as originally filed.

A substitute specification is included that provides different formatting and the introduction of sequence identifier labels where additionally needed to conform to standard U.S. practice.

It is thus seen that no new matter has been added by these amendments.

SUMMARY

The application is believed to be in condition for allowance. An early notice to that effect is earnestly solicited.

A filing fee is enclosed based on the number of independent and dependent claims in the application after entry of this Preliminary Amendment. No further fee or petition is believed to be necessary. However, should any further fee be needed, please charge our Deposit Account No. 23-0920, and deem this paper to be the required petition.

The Examiner is requested to phone the undersigned should any questions arise that can be dealt with over the phone to expedite this prosecution.

Respectfully submitted,


Shannon L. Nebolsky, Reg. No. 41,217

PTO Customer # 24628
WELSH & KATZ, LTD.
120 South Riverside Plaza
22nd Floor
Chicago, Illinois 60606
(312) 655-1500

CERTIFICATE OF EXPRESS MAILING

I hereby certify that this Preliminary Amendment including clean and marked-up copies of the Amendments, together with a § 371 application and its papers and fee, are being deposited with the United States Postal Service as Express Mail Label No. EL642288403US, postage prepaid, in an envelope addressed to: Commissioner for Patents, Box PCT, Washington, D.C. 20231 on November 8, 2001.


Shannon L. Nebolsky

methods described in the literature to extract DNA do not allow PCR amplification from these samples. Thus, we developed alternative methods for the extraction of DNA from these gums, which allowed DNA amplification. Using primers PG21 (SEQ ID NO:4) and PG22 (SEQ ID NO:6), we obtained amplification by PCR from the DNA extracted of all known samples of guar gum, and no amplification from any of control samples of locust bean gum. Furthermore, we demonstrate that with these DNA extraction and amplification methods we have detected guar DNA both in known control mixtures of guar and locust bean gum and in some commercial preparations labeled as "locust bean gum" which, theoretically, should not contain guar.

-Detailed description of the invention and examples.

[0011] As used herein, the term locust bean gum or E 410 is the ground endosperm of the seeds of the natural strains of carob tree *Ceratonia siliqua* [1998, "Commission Directive 98/86/EC of 11 November 1998," *Official Journal of the European Communities*, 9.12.98:L334/11]. The term guar gum or E 412, as used herein, is the ground endosperm of the seeds of natural strains of the guar plant [1998, "Commission Directive 98/86/EC of 11 November 1998," *Official Journal of the European Communities*, 9.12.98:L334/11]. For a detailed description of both terms we conform to what is described in the reference [1998, "Commission Directive 98/86/EC of 11 November 1998," *Official Journal of the European Communities*, 9.12.98:L334/11]. Although this invention does not claim taxonomical issues, some of the sequences described here as part of the claims may have taxonomical applications. Thus, the taxonomical status of carob tree and guar plant species requires further explanation. For example, in reference [1998, "Commission Directive 98/86/EC of 11 November 1998," *Official Journal of the European Communities*, 9.12.98:L334/11] both plant species are considered as belonging to the family *Leguminosae*, whereas other

Brief description of the drawings

[0023] **Figure 1A** shows the organization of the 18S-26S rDNA region. The relative positions of PCR primers used for the amplification of ITS1 and ITS2 containing regions, respectively the primer pairs ITS5 (SEQ ID NO:1)/ITS2 (SEQ ID NO:5) and ITS3 (SEQ ID NO:2)/ITS4 (SEQ ID NO:3), are shown. It also shows the position of the primers pair PG21 (SEQ ID NO:4)/PG22 (SEQ ID NO:6) used for the specific amplification of an internal region of the ITS2-containing region of guar. Sequences of primers ITS5 (SEQ ID NO:1), ITS2 (SEQ ID NO:5), ITS3 (SEQ ID NO:2), and ITS4 (SEQ ID NO:3) have been described in [White, T. J., T. Bruns, S. Lee, and J. Taylor, 1990, "Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics," in M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), PCR Protocols. A Guide to Methods and Applications, Academic Press, Inc., San Diego].

[0024] **Figure 1B** shows the results of the electrophoretic analysis in agarose gels of the PCR amplicons from DNA extracted from guar and carob tree seeds. Samples were amplified by PCR using the primer pairs ITS5 (SEQ ID NO:1)/ITS2 (SEQ ID NO:5) (gel lanes 1 and 2) and ITS3 (SEQ ID NO:2)/ITS4 (SEQ ID NO:3) gel (lanes 3 and 4) and therefore contain the ITS1 region and the ITS2 region, respectively. Carob tree amplicons are in lanes 1 and 3, and guar amplicons correspond to lanes 2 and 4. Lane St corresponds to DNA molecular mass markers, whose sizes (in nucleotides) are indicated on the left side of the figure. Samples were detected by electrophoresis on agarose gels, staining with SYBR® Gold Nucleic Acid Gel Stain (Molecular Probes) and UV observation.

[0025] **Figure 2** shows the sequences of the products shown on lanes 1 and 2 of Figure 1B, i.e., ITS1 containing regions of guar plant and carob tree. Products were sequenced using the ITS5 and ITS2 primers (boxed in the figure). Sequence names (identifiers) correspond to EMBL/GenBank/DDBJ DNA accession numbers. Sequence identifier AJ245575 (SEQ ID NO:8) corresponds to carob tree, and sequence identifier AJ245578 (SEQ ID NO:7) corresponds to guar. Sequences were aligned using the MacVector program, and dashes correspond to sequence gaps automatically introduced by the program to improve the alignment, whereas asterisks indicate nucleotide positions conserved in the two sequences. The ITS1 region (underlined) was determined by comparison to published sequences of several angiosperm plants [HersHKovitz, M. A., and E. A. Zimmer, 1996, "Conservation patterns in angiosperm rDNA ITS2 sequences," *Nucleic Acids Research*, 24:2857-2876].

[0026] **Figure 3** shows the sequences of the products shown on lanes 3 and 4 of Figure 1B, i.e., ITS2 containing regions of guar plant and carob tree. Products were sequenced using the ITS3 and ITS4 primers (boxed in the figure). Sequence identifiers are accession numbers of the EMBL/GenBank/DDBJ DNA databases. Sequence identifier AJ245576 (SEQ ID NO:10) corresponds to carob tree, and sequence identifier AJ245577 (SEQ ID NO:9) corresponds to guar. Sequences were aligned using the MacVector program, and dashes correspond to sequence gaps, whereas asterisks indicate conserved nucleotide positions. The ITS2 region (underlined) was defined by comparison to published sequences of several angiosperm plants [HersHKovitz, M. A., and E. A. Zimmer, 1996, "Conservation patterns in angiosperm rDNA ITS2 sequences," *Nucleic Acids Research*, 24:2857-2876]. The sequences of primers PG21 (SEQ ID NO:4) and PG22 (SEQ ID NO:6) designed in this patent are also shown.

on lanes 3 and 4 were obtained with the primers pair ITS3/ITS4, thus resulting in amplification of the rDNA regions containing ITS2.

[0035] The products visualized in Figure 1B were also sequenced on an Automated Applied Biosystems 373 DNA Sequencer using the fluorescent dye terminator procedure and the methods recommended by the supplier. Products were either directly sequenced or cloned in the pGEM[®]-T vector (Promega) and sequenced (in such cases, at least 3 clones of each amplification product were analyzed to establish the sequence). For sequencing, the following primers shown in Figure 1A were used: ITS5 (SEQ ID NO:1) and ITS2 (SEQ ID NO:5), for sequencing the ITS1 products of Figure 1B (lanes 1 and 2), and primers ITS3 (SEQ ID NO:2) and ITS4 (SEQ ID NO:3) for sequencing the ITS2 products of Figure 1B (lanes 3 and 4).

[0036] Figures 2 and 3 show the resulting sequences aligned with the MacVector program. Sequence identifiers (sequence names) correspond to accession numbers given by the EMBL/GenBank/DDBJ DNA databases. The boundaries of the ITS sequences were determined by comparison of our sequences with those of other angiosperm plants [HersHKovitz, M. A., and E. A. Zimmer, 1996, "Conservation patterns in angiosperm rDNA ITS2 sequences," *Nucleic Acids Research*, 24:2857-2876]. As shown in Figures 2 and 3 there are several nucleotide positions in the aligned sequences that are different between the guar plant and carob tree sequences. Also, there are differences in the length of the sequences obtained from the two plant species: the ITS1 containing region of the guar plant is 2 nucleotides longer than that of the carob tree, and the ITS2 containing region of the carob tree sequence is 5 nucleotides longer than that of the guar plant. These differences in sequence length would suffice by themselves to distinguish if the amplification products originate from the guar plant or the carob tree, using methods that are known in the molecular biology field.

[0040] The examples shown in Figures 4 and 5 are non-limiting: using the same methodology but with other restrictases that are commercially available it would be possible to produce differential restriction patterns from the DNA of the two plants under study. In this type of restriction analyses, a prediction of the usefulness of the different endonucleases can be performed by computer-assisted restriction analysis of the sequences, using MacVector or other computer programs.

Example III

Design and use of PCR primers specific of the DNA extracted from guar seeds

[0041] DNAs extracted from control seeds of guar and carob tree plants by the CTAB method (see example I) were amplified using primers PG21 (SEQ ID NO:4) and PG22 (SEQ ID NO:6) and the same PCR conditions detailed in example I, except that the annealing temperature was 55 °C. The amplification products were detected by electrophoresis on 1X TAE-3% agarose gels, and visualized by ethidium bromide or SYBR® Gold staining and UV observation.

[0042] Figure 6 shows the results of this type of experiments, and demonstrates that primers PG21 (SEQ ID NO:4) and PG22 (SEQ ID NO:6) amplify specifically the DNA extracted from guar and not from carob tree. The amplification fragment should have a length of exactly 194 nucleotides, as deduced from sequence AJ245577 (SEQ ID NO:9, Figure 3) in the region comprised between PG21 (SEQ ID NO:4) and PG22(SEQ ID NO:6). As shown in Figure 6, the fragment amplified from different guar seeds runs in the gels slightly below the 200 nucleotides marker, i.e., with the expected molecular mass.

Example IV

Extraction of DNA from guar and locust bean gums and their mixtures

[0043] DNA extraction from guar and locust bean gum and their mixtures was performed as follows. Ten mg of gums, obtained as powders from the suppliers, were suspended in 10 ml of either water (water method), 10 mM Tris-HCl pH 8.5 (Tris method), water: acetonitrile 7:3 (acetonitrile method), or 35% ethanol (ethanol method). Suspensions were vortexed for 5 min and centrifuged for 1 min at 15,000 x g. Supernatant fluids were recovered and used for PCR and quantitation assays, except in the ethanol method, where the procedure explained in the reference [Michaels, S. D., M. C. John, and R. M. Amasino, 1994, "Removal of polysaccharides from plant DNA by ethanol precipitation," *Biotechniques*, 17:274-276] was followed, and the final pellet obtained by this method was dissolved in 0.5 ml of water. For quantitation assays of the DNA extracted by the above four methods, 0.1 ml samples for DNA and the PicoGreen^R dsDNA Quantitation Kit of Molecular Probes were used. For total sugars quantitation, 0.01 ml of samples (extracts) and the phenol-sulfuric acid method [Keleti, G., and W. H. Lederer, 1974, Handbook of Micromethods in the Biological Sciences, Van Nostrand Reinhold, New York] were used. For PCR amplification, 0.005 ml of extracts were amplified with the PG21 (SEQ ID NO:4)/PG22 (SEQ ID NO:6) primers pair and the conditions described in Example III. The results obtained with these extraction methods are summarized in Table 1.

[0044] As shown in Table 1, the four assayed methods had different efficiencies in extracting the DNA from guar gum and locust bean gum. Clearly, the Tris method was the most efficient one, followed by the water, acetonitrile, and the ethanol methods. These efficiencies in DNA extraction do not always correlate with the amount of solubilized polysaccharide, as can be deduced from the Table column showing the DNA to polysaccharide ratios obtained for each solubilization method. When extracting individual gums (100% guar or locust bean gum), it is clear that the Tris method is, compared to the other methods, much more efficient for the extraction of DNA from locust bean gum than from guar gum. This can be deduced from the ratios of DNA extracted by each method from each gum: for locust bean gum, the amount of DNA extracted by Tris divided by the amount of DNA extracted by water is 35.8, whereas the same ratio for guar gum is 1.6.

[0045] The above results help to understand the results found when DNA extraction methods were assayed on locust bean gum with known additions (30% or 10%, w/w) of guar gum, and the results obtained when the DNAs extracted from the gums and their mixtures were assayed by PCR. Clearly, although the water method extracts less DNA than the Tris method from each gum or mixture assayed, the water method is the most adequate for detecting guar DNA extracted from guar gum or guar/locust bean gum mixtures. This is demonstrated in column labeled as "PCR" in Table 1, where the results of PCR amplification with the guar specific primers PG21 (SEQ ID NO:4)/PG22 (SEQ ID NO:6) are shown. Clearly, although the Tris method extracts more DNA than the water method from locust bean gum containing 30% or 10% guar gum, only the water method results in positive amplification and detection of the guar DNA contained in these mixtures. These extraction and PCR results suggest that most of the DNA extracted from gums mixtures using the Tris

solvent should actually correspond to locust bean DNA, rather than guar DNA, whereas with the water method sufficient guar DNA is extracted from guar/locust bean gum mixtures to allow its PCR amplification with the guar specific primers PG21 (SEQ ID NO:4)/PG22 (SEQ ID NO:6).

[0046] In summary, the results obtained advised to use the water method for the detection of guar gum in mixtures of guar gum and locust bean gum by the PCR methods described in this patent.

Example V

Specific detection of guar DNA in guar gum and in mixtures of guar gum and locust bean gum

[0047] In this example, laboratory preparations of locust bean gum containing different known amounts of guar gum and commercial locust bean gum samples were tested. DNA extraction by the water method described in the previous example and PCR amplifications with the PG21 (SEQ ID NO:4)/PG22 (SEQ ID NO:6) primers described in previous examples were used. Results of these studies are shown in Figures 7 and 8.

[0048] Figure 7 shows the results of testing laboratory mixtures of locust bean containing 30%, 20% and 10% of guar gum. These three mixtures were prepared from the same two samples of locust bean gum and guar gum. Reasoning that perhaps different gum manufactures may produce guar gums with different amounts of DNA, depending on their production processes or other reasons, we also prepared mixtures of the same locust bean gum plus a different guar gum. These locust bean gum mixtures contained 12%, 6% and 2% guar gum. As shown in Figure 7, all the locust bean gum mixtures containing guar gum produced by PCR with the PG21 (SEQ ID NO:4)/PG22 (SEQ ID NO:6) primers an amplification band of the expected size, independently of the amount of guar present in the mixture. Control samples containing only locust bean gum or only guar gum gave the expected results:

negative (no amplification by PCR) for the locust bean gum and positive (PCR amplification) for the guar gum. It is worth noting that, confirming our expectations, different commercial samples of guar gum contain different amounts of DNA. This can be deduced from the comparison of the results obtained, for example, from mixtures containing 2% and 10% guar gum (Figure 7 lanes 8 and 5, respectively). These two samples were prepared with the same locust bean gum but with guar gums from two different providers.

[0049] The usefulness of the methods described here for detecting guar gum was also tested on commercial preparations labeled as "locust bean gum". We obtained these commercial samples from different providers and selected some of them for study following the methods described in this patent. Particularly, we selected those samples producing viscosities higher than 250 cps at room temperature, since locust bean gum at this temperature usually produces viscosities not higher than 100 cps, and higher viscosities could be due to oversight or undeclared presence of guar gum. As shown in Figure 8, four out of the four theoretically pure locust bean gum preparations studied produced positive amplifications by PCR with the guar -specific primers pair PG21 (SEQ ID NO:4)/PG22 (SEQ ID NO:6) (lanes 2 through 5 in this Figure). This indicates that the four mentioned samples contained guar, although in different proportions, as can be deduced from the different intensities of the gel bands (compare for example lanes 2 and 3). Control gums produced the expected results: locust bean gum produced no amplification (lane 6), whereas guar gum amplified (lane 7). One commercial sample labeled as "locust bean gum" with the usual viscosity values of locust bean gum was also negative by PCR (lane 1).

[0050] An additional proof of the guar presence in some commercial preparations labeled as "locust bean gum" was obtained by restriction analysis of the amplification products mentioned in the last paragraph. For designing this analysis, we followed the directions and methods used in Example II and the AJ245577 (SEQ ID NO:9) sequence

shown in Figure 3. As shown in Figure 9, the amplification products seen in Figure 8 contained two restriction sites for endonuclease *TaqI*, which generates three fragments of 106, 52, and 36 nucleotides. The same enzyme would generate (as shown by computer-assisted restriction analysis) only two fragments of 143 and 52 nucleotides on the equivalent carob tree sequence, i.e., the sequence comprised between the PG21 (SEQ ID NO:4) and PG22 (SEQ ID NO:6) primers. Additionally, the *XhoI* enzyme, which does not cut the carob tree sequence, produces two fragments in the amplicons, as expected from the guar sequence comprised between PG21 (SEQ ID NO:4) and PG22 (SEQ ID NO:6) sequences. Thus, the enzymes used in this example are diagnostic, but also other enzymes (as discussed in Example II) can be used for the same purpose.

Claims

1. ~~Methods for differentiating the seeds of the plant species *Cyamopsis tetragonolobus* (or *Cyamopsis tetragonoloba*) and *Ceratonia siliqua*, said methods based on confirming the differences in the rDNA extracted from the seeds of these two species following these steps:~~
 - ~~i) the seeds are germinated and DNA is extracted from them;~~
 - ~~ii) the extracted DNA are amplified by PCR with primers ITS2, ITS3, ITS4, and ITS5 that are specific of the rDNA, and that are used in combinations as ITS5/ITS2 or ITS3/ITS4 pairs, and~~
 - ~~iii) the rDNA amplification products are detected~~
2. ~~Methods for detecting the differences in the rDNA amplified from the seeds using the methods described on claim 1, said methods comprising sequencing of the rDNA products of amplification and comparison with the guar plant and carob tree rDNA sequences described in this patent. The degree of identity of the sequence obtained from an unknown seed with those of the guar plant or carob tree allows deduction of its correspondence to one of these two species. The sequences of carob tree are AJ245575 and AJ245576, and those of the guar plant are AJ245577 and AJ245578.~~
3. ~~Methods for detecting the differences in the rDNA amplified from seeds using the methods described on claim 1, said methods not requiring DNA sequencing and that, taking advantage of the differences in the sequences described on claim 2, identify the amplified rDNA as arising from carob tree or guar. In these methods, the rDNA products of amplification are:~~
 - ~~i) digested with restriction endonucleases that produce different fragments according to the sequence being digested, such as, but not exclusively, the enzymes *BclI*, *ClaI*, *HaeIII*, *XhoI* or *SmaI*,~~

- ~~ii) the digestion products are resolved by, for example, electrophoresis in agarose gels and visualized by staining with SYBR® Gold Nucleic Acid Gel Stain (Molecular Probes), ethidium bromide or other DNA detection reagents, and~~
- ~~iii) the products of the digestion resolved in the gels are compared to those obtained from control guar and carob tree, so that the coincidence with the restriction patterns of the rDNA amplified from one or the other plant species allows the attribution of the rDNA amplified and digested from an unknown seed to one of the two cited plant species.~~

~~4. Methods for the specific detection of the guar seeds based on the rDNA sequences of guar and carob tree described in claim 2, said method comprising the steps:~~

- ~~i) these sequences are compared by alignment, and primers directed against the guar sequences are designed. These primers are identical or similar to sequences of the guar plant but are different in the carob tree sequence, such as the PG21 and PG22 sequences described in this patent.~~
- ~~ii) said oligonucleotides are used as primers for the amplification by PCR of the DNAs extracted from the seeds of guar, carob tree or from unknown seeds suspected to belong to one of these two species, and~~
- ~~iii) the DNA products of amplification are detected by any of the methods described on claims 2 and 3.~~

~~5. Methods for detecting guar gum (E 412) alone or mixed with locust bean gum (E 410), said methods comprising DNA extraction from these gums or their mixtures, amplification by PCR and detection of the amplification products corresponding to guar.~~

~~6. Methods for the extraction of DNA from guar gum (E 412) and locust bean gum (E 410) and their mixtures, comprising the steps of:~~

- ~~i) a certain amount of these gums or their mixtures is weighed and suspended in a certain volume of, preferably, water, or a buffered or unbuffered aqueous solution, containing or not other components like acetonitrile or ethanol,~~
- ~~ii) the suspension is shaken for a variable time, preferably 5 minutes, at a temperature between 0 °C and 100 °C, preferably at room temperature,~~
- ~~iii) the solubilized materials are separated by decantation or preferably by centrifugation, for example at 15,000 x g for 1 minute, and~~
- ~~iv) a sample of the soluble material, i.e., the upper phase of the decantation or centrifugation step, is recovered for its amplification by PCR.~~

~~7. Methods for the amplification by PCR and detection of the amplification products of the DNAs extracted from guar gum, locust bean gum, and mixtures of these gums, following the methods of claim 6 and characterized by the use of the methods described on claims 2, 3, and 4.~~

8. A method for differentiating the seeds of the plant species of *Cyamopsis tetragonolobus* and *Ceratonic siliqua* from each other or other seeds based on their different rDNA, said method comprising the steps of:

- i) germinating seeds of a plant to form germinated seeds;
- ii) extracting DNA from the germinated seeds to form extracted DNA;
- iii) amplifying the extracted DNA using primers ITS2 (SEQ ID NO:4), ITS3 (SEQ ID NO:2), ITS4 (SEQ ID NO:3) and ITS5 (SEQ ID NO:1) to form rDNA amplification products; and
- iv) detecting the rDNA amplification products, thereby differentiating the seeds of the plant species of *Cyamopsis tetragonolobus* and *Ceratonic siliqua* from each other or other seeds.

9. The method according to claim 8 wherein said primers are one or more of the pairs ITS5/ITS2 (SEQ ID NO:1/SEQ ID NO:4) and ITS3/ITS4 (SEQ ID NO:2/SEQ ID NO:3).

10. The method according to claim 8 further comprising the steps of:

- v) sequencing the rDNA amplification products; and
- vi) comparing the sequenced rDNA to one or more of carob tree sequence AJ245575 (SEQ ID NO:8), carob tree sequence AJ245576 (SEQ ID NO:10), guar plant sequence AJ245577 (SEQ ID NO:9) and guar plant sequence AJ245578 (SEQ ID NO:7).

11. The method according to claim 9 further comprising the steps of:

- v) digesting the rDNA amplification products with a restriction endonuclease to form restriction fragments of the rDNA;
- vi) resolving the restriction fragments of the rDNA; and
- vii) comparing the resolved restriction fragments of the rDNA to restrictions fragments from one or more of control guar and carob tree DNA digested with the same restriction endonuclease.

12. The method according to claim 11 wherein said restriction endonuclease is selected from the group consisting of: *BcnI*, *ClaI*, *HaeIII*, *XhoI* and *SmaI*.

13. The method according to claim 11 wherein the restriction fragments of the rDNA are resolved by electrophoresis in agarose gels.

14. The method according to claim 13 wherein the resolved digestion products are visualized by staining with a DNA detection reagent selected from the group consisting of: ethidium bromide and a fluorescent nucleic acid gel stain.

15. A method for specifically distinguishing guar seeds from other seeds, said method comprising the steps of:

- i) germinating seeds of a plant to form germinated seeds;
- ii) extracting DNA from the germinated seeds to form extracted DNA;
- iii) preparing guar-specific primers that are identical to a portion of guar plant sequence AJ245577 (SEQ ID NO:9) or AJ245578 (SEQ ID NO:7) but different from portion of carob tree sequence AJ245575 (SEQ ID NO:8) or AJ245576 (SEQ ID NO:10) that aligns with the portion of guar plant sequence

- iv) amplifying the extracted DNA from step ii using the guar-specific primers from step iii to form rDNA amplification products; and
- v) detecting the rDNA amplification products, thereby specifically distinguishing guar seeds.

16. The method according to claim 15 wherein said guar-specific primers are PG21 (SEQ ID NO:4) and PG22 (SEQ ID NO:6).

17. A method for detecting the presence of guar gum (E 412) alone or mixed with locust bean gum (E 410) in a gum sample, said method comprising the steps of:

- i) extracting DNA from a gum sample;
- ii) amplifying the DNA using guar-specific primers that are identical to a portion of guar plant sequence AJ245577 (SEQ ID NO:9) or AJ245578 (SEQ ID NO:7) to form amplified DNA;
- iii) detecting the amplification products in the amplified DNA that are specific to guar.

18. A method for obtaining extracted DNA from gum samples comprising one or more of guar gum (E 412) and locust bean gum (E 410), comprising the steps of:

- i) contacting a gum sample comprising DNA and one or more of guar gum (E 412) and locust bean gum (E 410) with an aqueous solution to form an extraction mixture;
- ii) agitating the extraction mixture at a temperature between 0°C and 100°C for a time period sufficient to permit extraction of DNA from the gum sample into the aqueous solution;
- iii) separating the extraction mixture to obtain an aqueous solution containing extracted DNA and another phase; and
- iv) recovering a sample of the aqueous solution containing extracted DNA.

19. The method according to claim 18 wherein said aqueous solution is a buffered aqueous solution.

20. The method according to claim 18 wherein said aqueous solution further comprises acetonitrile or ethanol.

21. The method according to claim 18 wherein the extraction mixture is agitated at room temperature.

22. The method according to claim 18 wherein the extraction mixture is separated by decantation.

23. The method according to claim 18 wherein the extraction mixture is separated by centrifugation.

24. The method according to claim 23 wherein the centrifugation is at 15,000 x g.

25. The method according to claim 18 further comprising the step of amplifying the extracted DNA using PCR.

26. The method according to claim 25 wherein said amplification utilizes one or more primers having a sequence that is SEQ ID NO:4, SEQ ID NO:6, a portion of SEQ ID NO:7 or a portion of SEQ ID NO:9.